In the Claims

Claim 1 (Previously presented): A method for increasing the number of polynucleotides containing sequences corresponding to a mRNA species present in a sample, the method comprising the steps of:

(i) in a single step, reverse transcribing the mRNA species using a heeled 5'-amplification primer (FAP-RAND) and a heeled 3'-amplification primer (TAP-RT), wherein each heeled primer comprises a heel sequence that includes a unique sequence, and either or each heel sequence includes a RNA polymerase promoter site, and the heeled 5'-amplification primer includes a variable sequence, whereby the RNA is reverse-transcribed to produce double-stranded cDNA and then multiple cDNAs according to the variable sequence; and

(ii) amplifying the cDNA using primers sufficiently complementary to the unique sequences within the heel sequences of the heeled 5'-amplification primer and the heeled 3'-amplification primer.

Claim 2 (Previously presented): The method according to claim 1, which additionally comprises the step of:

(iii) *in vitro* transcribing, to produce RNA run-offs from either end of the amplicons.

Claim 3 (Previously presented): The method according to claim 1, wherein each heel sequence includes a different RNA polymerase site.

Claim 4 (Previously presented): The method according to claim 3, for the production of a strand-specific library.

Claim 5 (Previously presented): The method according to claim 1, for the production of a subtracted library from two cell populations.

Claim 6 (Previously presented): The method according to claim 1, which further comprises cloning the polynucleotide products and immobilizing them in an array.

Claim 7 (Previously presented): The method according to claim 1, wherein the sample is from laser capture microdissection.

Claim 8 (Previously presented): The method according to claim 1, wherein the sample is from patch clamp harvesting.

Claim 9 (Previously presented): The method according to claim 1, wherein the heeled 5'-amplification primer heel sequence or the heeled 3'-amplification primer heel sequence, or both, includes the nucleotide sequence of a cleavage site.

Claim 10 (Previously presented): The method according to claim 9, wherein the cleavage site is located at the 3' end of its heel sequence.

Claim 11 (Previously presented): The method according to claim 10, wherein the heeled 5'-amplification primer and the heeled 3'-amplification primer have identical cleavage sites.

Claim 12 (Previously presented): The method according to claim 10, wherein the heeled 5'-amplification primer and the heeled 3'-amplification primer have different cleavage sites.

Claim 13 (Previously presented): The method according to claim 9, which comprises the additional step of treating the polynucleotides with an agent that cleaves at the cleavage site.

Claim 14 (Previously presented): The method according to claim 1, wherein said amplifying comprises up to 50 amplification cycles.

Claim 15 (Previously presented): The method according to claim 14, wherein each amplification cycle comprises the steps of:

(i) obtaining single-stranded DNA molecules at a temperature between 85°C and 97°C;

(ii) annealing the single-stranded DNA molecules at a temperature between 45°C and 65°C; and

(iii) elongating the annealed DNA molecules at a temperature between 70°C and 75°C.

Claim 16 (Previously presented): The method according to claim 1, wherein the heeled 5'-amplification primer population consists of a population of nucleic acids comprising, from 5' end to 3' end:

(i) a heel sequence, of 15 to 22 nucleotides, which is not complementary to the mRNA molecules initially present in the sample; and

(ii) an oligo dT sequence of 15 to 25 nucleotides;

wherein substantially every possible variable sequence combination is found in said heeled 5'-amplification primer population.

Claim 17 (Previously presented): The method according to claim 1, which additionally comprises confirming the presence of at least one nucleic acid sequence contained in the reaction mixture after said amplifying.

Claim 18 (Previously presented): The method according to claim 17, wherein said confirming comprises any of the following methods:

(i) detecting sequences of interest with specific oligonucleotide probes;

(ii) amplifying sequences of interest with specific oligonucleotide primers; and

(iii) cloning DNA molecules obtained in a replication or expression vector.

Claim 19 (Previously presented): The method according to claim 2, wherein each heel sequence includes a different RNA polymerase site.

Claim 20 (New): The method according to claim 2, wherein the RNA run-offs are produced using T3 polymerase or T7 polymerase.